Form PTO-1390U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 10-95) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER **2364-011622**
		U.S. APPLICATION NO. (If known, see 37 CFR 1 5) **10/009226**
INTERNATIONAL APPLICATION NO. **PCT/DE00/01404**	INTERNATIONAL FILING DATE **03.05.00 (03 May 2000)**	PRIORITY DATES CLAIMED **20.05.99 (20 May 1999)**

TITLE OF INVENTION **METHOD FOR DIAGNOSING TSE-INDUCED CHANGES IN TISSUES USING INFRARED SPECTROSCOPY**

APPLICANT(S) FOR DO/EO/US **Dieter NAUMANN, Janina KNEIPP, Elizabeth BALDAUF, Peter LASCH and Michael BEEKES**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S C. 371.

3. ☒ This express request to begin national examination procedures (35 U S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English (35 U.S C 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:
    a. WO 00/72007-Front Page and International Search Report (5 pp.)

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/009226	INTERNATIONAL APPLICATION NO. PCT/DE00/01404	ATTORNEY'S DOCKET NUMBER 2364-011622

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Search Report has been prepared by the EPO or JPO..... **$890.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) **$710.00**

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))..... **$740.00**

Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **$1040.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... **$100.00**

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20 - 20	0	X **$18.00**	$ 0.00	
Independent claims	1 - 3 =	0	X **$84.00**	$ 0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1020.00	
Reduction of 1/2 for filing by small entity, if applicable. Small Entity Status verified by applicant's attorney.				$ 510.00	
SUBTOTAL =				$ 510.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
TOTAL NATIONAL FEE =				$ 510.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0.00	
TOTAL FEES ENCLOSED =				$ 510.00	
				Amount to be: Refunded	$
				Charged	$

a. ☒ A check in the amount of $ **510** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. ____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Assistant Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-0650. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
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Richard L. Byrne
SIGNATURE
Richard L. Byrne
NAME
28,498
REGISTRATION NUMBER

PATENT APPLICATION/PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	ATTORNEY'S DOCKET NUMBER
Dieter NAUMANN **Janina KNEIPP** **Elizabeth BALDAUF** **Peter LASCH** **Michael BEEKES**	**2364-011622**

PCT/DE00/01404

ENTITLED

METHOD FOR DIAGNOSING TSE-INDUCED CHANGES IN TISSUES USING INFRARED SPECTROSCOPY

To **BOX PCT**
Attention: DO/EO/US

Commissioner for Patents
Washington, D.C. 20231

EXPRESS MAIL CERTIFICATE

"Express Mail" Label Number **EL561554302US**

Date of Deposit November 8, 2001

I hereby certify that the following attached paper or fee

Transmittal Letter To The United States Designated/Elected Office (DO/EO/US) Concerning A Filing Under 35 U.S.C. 371 (original and two (2) copies) And Check In The Amount Of $510.00 For Filing Fee; Letter Recognizing Attorneys (2 pp.); Preliminary Amendment; WO 00/72007 Front Page (1 p.) And International Search Report (4 pp.); Certification Of Verification (1 p.); Specification (13 pp.); Claims (3 pp.); Five Sheets Of Drawings

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

K.T. Berthold
(Typed name of person mailing paper or fee)

K.T. Berthold
(Signature of person mailing paper or fee)

PATENT APPLICATION/PCT
Attorney Docket No. 2364-011622

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	
Dieter NAUMANN	:	METHOD FOR DIAGNOSING
Janina KNEIPP	:	TSE-INDUCED CHANGES IN TISSUES
Elizabeth BALDAUF	:	USING INFRARED SPECTROSCOPY
Peter LASCH	:	
Michael BEEKES	:	
International Application	:	
No. PCT/DE00/01404	:	
International Filing Date	:	
03 May 2000	:	
Priority Date Claimed	:	
20 May 1999	:	
Serial No. Not Yet Assigned	:	
Filed Concurrently Herewith	:	

Pittsburgh, Pennsylvania
November 8, 2001

PRELIMINARY AMENDMENT

Box PCT
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Please insert and delete section headings and amend specification paragraphs as follows. (Pursuant to 37 CFR 1.121, marked-up versions of the amended specification paragraphs are attached.)

On page 1, before the title, please delete the section heading "Description".

On page 1, after the title, at line 6, please insert the following section headings:

BACKGROUND OF THE INVENTION

1) Field of the Invention

Before the paragraph beginning at page 1, line 12, please insert the following section heading:

2) Brief Description of the Prior Art

Before the paragraph beginning at page 4, line 1, please insert the following section heading:

SUMMARY OF THE INVENTION

Please replace the paragraph beginning at page 4, line 1, with the following rewritten paragraph:

It is thus the problem of this invention to provide a method for detecting TSE-induced pathologic changes in tissues. Said changes may be caused by scrapie, BSE, or any other of the TSE diseases. This problem is solved according to the invention by (a) directing infrared radiation to a tissue sample with pathologic changes caused by TSE, recording its spectral characteristics after irradiation and (b) comparing and classifying the infrared spectra thus obtained with a reference database containing infrared spectra of TSE-infected tissues and non-infected tissues.

Before the paragraph beginning at page 4, line 13, please insert the following section headings and specification paragraphs:

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flowchart of the method according to the present invention;

Fig. 2 is, in a first example of the present invention, a chart of two typical spectra of S- and N-tissue samples;

Fig. 3 is, in a second example of the present invention, a dendrogram of a specific hierarchical spectrum classification as calculated using Ward's algorithm;

Fig. 4A is, in a third example of the present invention, a dendogram calculated after data compression using principal component analysis; and

Fig. 4B, in the third example of the present invention, compares normed vector second derivations of sample spectra and differential spectra of normed vector S- and N-spectra.

DETAILED DESCRIPTION OF THE INVENTION

IN THE CLAIMS:

Please cancel claims 1-10 and rewrite them as new claims 11-30 as follows:

11. A method for diagnosing TSE-induced pathologic changes in tissues, said changes being caused by scrapie, BSE or another disease of the TSE group of diseases, comprising the steps of:

(a) directing infrared radiation onto a tissue sample showing pathologic changes due to TSE, and that the spectral characteristics after interaction with the sample are recorded; and

(b) comparing and classifying the infrared spectra thus obtained against a reference database that contains infrared spectra of TSE-infected and non-infected tissues.

12. The method according to claim 11, wherein said tissue sample is collected from one of the central nervous system, the peripheral nervous system and human organs.

13. The method according to claim 11, wherein said infrared spectrum of the tissue is measured in at least one region of one of the mid-infrared range from 500 to 4000 cm^{-1} and the near infrared range from 4000 to 1000 cm^{-1}.

14. The method according to claim 11, wherein said infrared spectrum of the tissue is measured in the spectral region from 10000 to 1300 cm^{-1} of the mid-infrared range.

15. The method according to claim 11, wherein said infrared radiation interacts with said sample, and the characteristically altered radiation is detected in one of a transmission/absorption, attenuated total reflection, direct reflection measuring setup, diffuse reflection measuring setup, and by using IR waveguides.

16. The method according to claim 11, wherein said infrared spectrum of the sample to be examined is compared against the reference database using at least one method of pattern recognition, and that the spectral regions said comparison is based on are determined using methods for extracting optimum spectral characteristics.

17. The method according to claim 11, wherein said infrared spectrum is measured on a thin slice of tissue using an IR microscope set up for one of transmission and direct reflection spectrometry.

18. The method according to claim 17, wherein said infrared spectra are measured in positional resolution.

19. The method according to claim 17, wherein each mapped infrared spectrum is compared against the reference database, thereby providing localized information on the spread of the disease in the tissue.

20. The method according to claim 17, wherein said reference database contains reference spectra of TSE-infected tissues and non-infected tissues of all structures that can be distinguished in the tissue section using infrared spectroscopy.

21. The method according to claim 11, wherein the human organs are from one of the lymphatic system, the digestive system, the endocrine system, the cardiovascular system and the respiratory system.

22. The method according to claim 16, wherein the at least one pattern recognition method uses algorithms of one of multivariate statistics and artificial neuronal networks.

23. The method according to claim 16, wherein the extracting optimum spectral characteristic method uses genetic algorithms.

24. The method according to claim 17, wherein said infrared spectra are mapped to the tissue site where the infrared beam is transmitted through the sample.

25. The method according to claim 24, wherein each mapped infrared spectrum is compared against the reference database, thereby providing localized information on the spread of the disease in the tissue.

26. The method according to claim 12, wherein said infrared spectrum of the tissue is measured in at least one region of one of the mid-infrared range from 500 to 4000 cm^{-1} and the near infrared range from 4000 to 10000 cm^{-1}.

27. The method according to claim 12, wherein said infrared spectrum of the tissue is measured in the spectral region from 1000 to 1300 cm^{-1} of the mid-infrared range.

28. The method according to claim 13, wherein said infrared spectrum of the tissue is measured in the spectral region from 1000 to 1300 cm^{-1} of the mid-infrared range.

29. The method according to claim 12, wherein said infrared radiation interacts with said sample, and the characteristically altered radiation is detected in one of a transmission/absorption, attenuated total reflection, direct reflection measuring setup, diffuse reflection measuring setup, and by using IR waveguides.

30. The method according to claim 13, wherein said infrared radiation interacts with said sample, and the characteristically altered radiation is detected in one of a transmission/absorption, attenuated total reflection, direct reflection measuring setup, diffuse reflection measuring setup, and by using IR waveguides.

IN THE ABSTRACT:

After the claims, please insert a page containing the Abstract Of The Disclosure, which is attached hereto as a separately typed page.

The specification and claims have been amended to place the application in conformance with standard United States patent practice.

Examination and allowance of pending claims 11-30 are respectfully requested.

Respectfully submitted,

WEBB ZIESENHEIM LOGSDON
ORKIN & HANSON, P.C.

By _____

Richard L. Byrne
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Paragraph beginning at page 1, line 14, has been amended as follows:

It is thus the problem of this invention to provide a method for detecting TSE-induced pathologic changes in tissues. Said changes may be caused by scrapie, BSE, or any other of the TSE diseases. This problem is solved according to the invention by (a) directing infrared radiation to a tissue sample with pathologic changes caused by TSE, recording its spectral characteristics after irradiation and (b) comparing and classifying the infrared spectra thus obtained with a reference database containing infrared spectra of TSE-infected tissues and non-infected tissues. [Embodiments of this invention are described in the subclaims.]

PATENT APPLICATION/PCT
Attorney Docket No. 2364-011622

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	
Dieter NAUMANN	:	METHOD FOR DIAGNOSING
Janina KNEIPP	:	TSE-INDUCED CHANGES IN TISSUES
Elizabeth BALDAUF	:	USING INFRARED SPECTROSCOPY
Peter LASCH	:	
Michael BEEKES	:	
International Application No. PCT/DE00/01404	:	
International Filing Date 03 May 2000	:	
Priority Date Claimed 20 May 1999	:	
Serial No. Not Yet Assigned	:	
Filed Concurrently Herewith	:	

Pittsburgh, Pennsylvania
November 8, 2001

LETTER RECOGNIZING ATTORNEYS

Box PCT
Commissioner for Patents
Washington DC 20231

Sir:

Enclosed are appropriate papers for initiating the national phase of the above-identified PCT application, comprising a specification, claims, abstract and drawings. A Preliminary Amendment is also enclosed.

Please accept the application for purposes of granting a filing date and recognize Richard L. Byrne, Russell D. Orkin and Nathan J. Prepelka, Registration Nos. 28,498, 25,363 and 43,016, respectively, as attorneys in this application, pending the filing of a formal Declaration and Power of Attorney.

Kindly direct all communications relating to this application to **Richard L. Byrne**.

Respectfully submitted,

WEBB ZIESENHEIM LOGSDON
ORKIN & HANSON, P.C.

By _____

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CERTIFICATE OF VERIFICATION

I, Dr. Wolf Grosskopf, resident at 2013 Los Feliz Drive Apt. 5, Thousand Oaks, CA 91362, a professional interpreter and translator sworn at Landgericht Berlin, member of tekom, Gesellschaft für technische Kommunikation e.V. (Society for Technical Communication), Eberhardstrasse 69-71, 70173 Stuttgart, Federal Republic of Germany, do hereby certify that I am well acquainted with the English and German languages and that to the best of my knowledge and belief the attached is a true translation into the English language of PCT application No. PCT/DE00/01404.

Dated this 26th day of September, 2001

Signature of translator:

Dr. Wolf Grosskopf
Für die Berliner Gerichte und Notare
allgemein beeidigter Dolmetscher
für Englisch und Russisch

Translator's stamp reads: Sworn translator for English and Russian at the courts of Land Berlin

5/prts

Description

A method for diagnosing TSE-induced changes in tissues using infrared spectroscopy

This invention relates to a method for fast detection of pathologic changes induced by transmissible spongiform encephalopathies (TSE) in animal or human tissue using infrared spectroscopy (IR spectroscopy).

Transmissible spongiform encephalopathies are communicable neurodegenerative diseases of the central nervous system (CNS) that may affect many mammals and humans. TSE is used as a cover term here that refers to the various forms of this disease as they occur in the various species. In addition to scrapie (trotting disease), the disease that originated in sheep but can be transmitted to hamsters and mice, five other types of TSE have become known as yet: Bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in some American deer and elk, transmissible mink encephalopathy (TME) in mink, feline spongiform encephalopathy (FSE) in cats, and a spongiform encephalopathy in antelopes. Four types of TSE are distinguished in humans: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), and kuru.

TSE can definitely be diagnosed based on a) histological proof of characteristic spongiform changes in the brain tissue accompanied by gliosis, b) immunological proof of deposits of the pathologic prion protein (PrP) using a Western blot test, histo-blot test, and immunohistochemistry, c) proof of scrapie-associated (PrP) fibrils (SAF) using an electron microscope, and d) proof of the infectious TSE agent using transmission experiments in animals.

Clinical symptoms and chemical laboratory findings of increased concentrations of specific proteins in cerebrospinal fluid and/or serum [Protein 14-3-3 (Zerr et al. (1997) *N. Engl. J. Med.* 336: 874; Zerr et al. (1998) *Ann. Neurol.* 43: 32-40.), Protein S100 (Otto et al. (1997) *J. Neurol.* 244: 566-570; Otto et al. (1998) *Brit. Med. J.* 316: S77S82; Otto et al. (1998) J. *Neurovirol.* 4: 572-573) as well as neuron-specific enolase (Zerr et al. (1995) Lancet 345: 1609-1610)] in animals and humans can only give rise to a tentative diagnosis. The same applies to changes visible in EEGs or MR tomograms that occur in conjunction with human TSE.

Development and improvement of detection procedures for TSE serve, inter alia, the following purposes.

a) Improving differential diagnostics of human TSEs. These diseases can only be diagnosed with any certainty by a post mortem or cerebral biopsy.

b) Detection of TSE contamination in blood, organs, and tissue and in products of human or animal origin produced from these.

c) Identification of blood, organ, and tissue donors infected with human TSE.

d) Detection of preclinical or clinical stages of TSE infection in farm animals (e.g. cattle and sheep) at the slaughterhouse or farm.

To be able to diagnose TSE diseases in farm animals is important because these diseases may be transmitted through eating the meat of diseased animals. It is suspected, for example, that the consumption of BSE-contaminated beef can cause a new variant of CJD in humans (nvCJD). Some states are currently introducing official monitoring of contamination levels in cattle populations to protect consumers and contain the spread

of the epidemic. It is envisaged to carry out routine checks at slaughterhouses to establish whether the carcasses can be used.

Various test systems are being developed to provide sensitive and fast screening of large sample populations for pathologic prion protein and thus to provide diagnostics for large-scale production. These include a capillary electrophoresis immunoassay using fluorescence-labeled peptides (Schmerr & Jenny (1998) *Electrophoresis* 19: 409-419) and an immunological detection system using fluorescent lanthanide chelates called Delfia (Safar et, al. (1998) Nature *Medicine* 4. 1157-1165).

Only one diagnostic method is currently available to identify TSE-infected farm animals that is suitable for large-scale application. It is restricted to use at slaughterhouses and, according to the developer's statements, can detect BSE in cattle up to half a year before clinical symptoms occur (see the manufacturer's information on the Internet at *http://www.prionics.ch*).

In this method developed by Swiss-based Prionics AG, a tissue sample obtained from the medulla oblongata of slaughtered cattle is homogenized and treated with proteinase K enzyme. The pathologic prion protein that may remain after this treatment is labeled with the 6H4 monoclonal antibody (manufactured by Prionics) and then stained using the Western blot method. The manufacturer states that it takes up to 12 hours from taking the sample to getting a final result using this method. It is the problem of this invention to develop a method that provides fast, reliable and cost-efficient detection of TSE-induced tissue changes. The method according to the invention is to work efficiently in the routine operations of a slaughterhouse.

It is thus the problem of this invention to provide a method for detecting TSE-induced pathologic changes in tissues. Said changes may be caused by scrapie, BSE, or any other of the TSE diseases. This problem is solved according to the invention by (a) directing infrared radiation to a tissue sample with pathologic changes caused by TSE, recording its spectral characteristics after irradiation and (b) comparing and classifying the infrared spectra thus obtained with a reference database containing infrared spectra of TSE-infected tissues and non-infected tissues. Embodiments of this invention are described in the subclaims.

The method used in this invention is mainly based upon measurements of infrared spectra of pathologically changed tissue. It has been known from a number of publications and patent applications that disease-specific changes may be reflected in the infrared spectrum of tissues (U.S. Patent 5,168,162 (Wong & Rigas; U.S. Patent 5,038,039; Wong, Rigas; Lasch & Naumann (1998) *Cell. Mol. Biol.* 44:189-202; Lasch et al. (1998) *Proc. SPIE* 3257: 187-198; Choo et.al. (1996) *Biophys. J.* 71: 1672-1679). However, no data have been published on infrared spectroscopy performed on TSE tissue samples.

The experimental data this patent description is based on was developed using CNS samples of scrapie-infected hamsters as a model system. It was found in this hamster model that characteristic changes in the infrared spectrum of CNS tissue samples occurred after the animals were infected with scrapie. These changes were identified using the method according to the invention, i.e. by comparing respective samples obtained from non-infected healthy animals. In principle, this method can be used for the diagnosis of any clinical picture of the TSE group of diseases. According to the method of the invention, TSE diagnosis using infrared spectrography requires comparing spectra of the tissue to be examined with spectra of tissues of known origin for reference. Practical application of this method therefore requires the existence of a validated

reference database of IR spectra obtained from healthy and pathologic tissue samples. This reference database has to be created just once to perform a standardized diagnostic method.

Matching spectra obtained from unknown samples with the reference database can be performed using methods of computer-aided pattern recognition such as multivariate statistics, artificial neuronal networks, genetic algorithms, etc.

The spectra are obtained by directing infrared light onto the samples and recording the spectral characteristics of the emerging radiation, i.e. after the light has interacted with the tissue. Use of microspectrometric techniques is advantageous when minimization of sample sizes is desired. When an infrared microscope is used, spectral data can be obtained from thin slices of tissue in positional resolution, which makes the method considerably more specific and sensitive. In future improvements, an infrared optical waveguide could be used as an endoscope and facilitate TSE diagnosis directly in the infected organism.

A typical flowchart of the method is shown in Fig. 1. The new method of detecting TSE-induced changes in tissue enables users to make statements within one minute from obtaining the sample. This makes it superior to immunological detection of the prion protein and immunohistological diagnosis, as these provide results after up to 12 hours only. So virtually no intermediate storage of the carcasses is required to wait for test results when the method according to the invention is used during routine operation. Fastness of this diagnostic method represents an economic advantage as compared to known methods because it minimizes the storage time of the carcasses and thus space and energy costs required for refrigerating them. In addition, the meat is fresher at the time of final consumption.

This method can easily be integrated into a routine process as the steps of recording, processing, and classifying the spectrum are fully computer-controlled and easily automated. Just a few staff members are required for the relatively simple process of sample preparation, but unlike other methods, pretreatment of the samples does not require a major effort (such as enrichment of the prion protein by proteinase K digestion) or staining thin slices of tissue (by immunohistological methods).

The method according to the invention can be performed without involving highly specialized professionals (such as histologists) as the IR spectra are classified using generally known methods of computer-aided pattern recognition that are optimized for the purposes of TSE diagnosis. As the spectra are evaluated according to strict mathematical criteria, diagnosis is highly reliable, does not require inference from experience, thus bypassing human misjudgment.

The method of the invention has an economically reasonable design due to its moderate staffing requirements and virtually no material costs during operation.

The advantage of the method according to the invention in its specific IR microscopy embodiment for analyzing thin slices of tissue in positional resolution is that it combines structural information in spectral resolution with the high positional resolution obtained from it. Involvement of individual neurons in the pathogenesis can be recorded and studied using the mapping an IR microscope can provide. The very high diagnostic sensitivity of the method results from the fact that characteristics of diseased and healthy cells are practically not averaged, which is bound to happen with methods that do not offer mapping functionality. This specific embodiment of the method still required relatively much time for data collection, which may take from 1 to 6 hours depending on the size of the tissue area under review and the positional reso-

lution. However, it should be widely used in scientific and clinical studies of the pathogenic mechanisms of TSE which have not yet been understood. In the future, this embodiment will be combined with so-called array infrared detectors that are being developed by various manufacturers and will be capable of measuring IR spectra of complete thin slice areas in positional resolution and in a very short time, making this method eventually suitable for fast routine diagnosis.

The method of the invention first requires taking *post mortem* tissue samples from the organism. Samples may be taken from animal and human organisms.

The method is suitable for detecting each of the special clinical forms that are covered by the term 'transmissible spongiform encephalopathy' (TSE), such as BSE, scrapie, or CJD.

All organs that show TSE-induced pathologic changes can be used as sites for collecting tissue samples. As far as we know today, affected organs are the central nervous system, the peripheral nervous system, organs of the lymphatic system, the digestive system, the endocrine system, the cardiovascular system, and the respiratory system. Preferred collection sites are the central nervous system and the peripheral nervous system, the medulla oblongata and the Varolian pons being particularly advantageous. The tissue sample is prepared depending on the specific way the method is carried out.

Small pieces of tissue are collected for analyzing fully hydrated tissue samples. The native samples are placed into commercial IR cuvets. Alternatively, a homogenizate of the tissue material in H_2O is produced, and aliquots are placed in IR cuvets. In a variation of the method, aliquots of this suspension are dried up as transparent films on IR-transmitting sample holders; the drying process is accelerated

by reduced pressure (Helm et al. (1991) *J. Gen. Microbiol.* 137:69-79).

Cryostat sections are made for carrying out the method in an IR microscopy measuring arrangement for collecting specific locally mapped data. These are evenly applied to IR transparent microscopic slides. The method does not require any fixing of the thin slice of tissue. The samples are stored at a dry place at room temperature until they are measured.

In another embodiment that uses infrared waveguides, the method can be applied in vivo by introducing the waveguide into the tissue using minimal invasion technique and directly collecting the infrared spectrum from there. This embodiment requires an improvement of infrared waveguide technology to become practicable as currently available waveguides still have a too low spectral sensitivity, are too inflexible and too big. Materials suitable for cuvets or sample holders/slides for the preparation variants described above are all water-insoluble optical materials conventionally used in IR spectroscopy, while CaF_2 and BaF_2 have proven particularly useful.

The amount of substance required for IR spectra and their superficial extent can be very small. Depending on the conditions set (such as spectroscopy with or without beam focusing or using an IR microscope), sample sizes in the µg to ng ranges can be used. The diameters of the irradiated sample areas vary between 1-3 mm and 10-30 µm. The lower limit is about the size of one or a few cells (e.g. neurons).

According to the method of the invention, the infrared spectra of tissue samples that were prepared in the way described are measured. The spectra are preferably taken using a Fourier transform infrared spectrometer which has a number of known advantages as compared to conventional disperse equipment, including fast data collection and higher sensitivity.

It would generally be possible to use a conventional disperse IR spectrometer but this would slow the method down. In principle, each of the generally known IR spectrometry setups (such as transmission/absorption, diminished total, direct, or diffuse reflection) can be used to measure the spectra. Transmission/absorption spectroscopy has proven particularly useful.

The infrared spectrum is typically taken in the mid-infrared spectral region, i.e. between 500 and 4000 cm^{-1}. Narrower spectral regions even in near infrared range from 4000 to 10000 cm^{-1} can also provide successful diagnosis if the user made sure that the spectra of infected and healthy tissue samples show characteristic variance in the spectral region recorded. It was found, in particular, that marked spectral differences between TSE-infected and non-infected tissues were detected in the range from 1000 to 1300 cm^{-1}, and that this range is particularly suited for diagnosis.

One or several suitable spectral regions can be selected by visual inspection of spectra (selecting the ranges that show the strongest and most characteristic changes as compared to the control group) or by a generally known multivariate method for selecting spectral characteristics.

The physical parameters such as spectral resolution or number of averaged spectra, etc. can be varied within the typical ranges in IR spectroscopy without having any critical practical influence on the success of classification or diagnosis. When determining the parameters for obtaining the spectra and preparing the sample, identical parameters have to be selected for all measurements including control measurements of tissue samples from non-infected animals.

Conditioning of the spectra has proven advantageous no matter which mathematical-statistical method was chosen for spectrum classification. The generally known methods that

can be used here include calculation of the first or second derivation, deconvolution, or other methods to increase spectral contrast, to facilitate band recognition, and to minimize any baseline problems that may occur. When sample populations are large, upfront data reduction using methods of multivariate statistics such as factor analysis has proven helpful.

The method requires one-time creation of a database of reference spectra. Spectra of samples from TSE-infected organisms and from non-infected individuals are measured. Samples are prepared and spectra taken in the same way as with unknown samples. It is important that all parameters for reference and sample measurements are identical.

The spectrum of the sample to be examined is compared with the spectra stored in the reference database. The spectrum is preferably classified using a method of pattern recognition such as algorithms of multivariate statistics, artificial neuronal networks, or genetic algorithms. This step classifies a spectrum based on a two-class problem as being either healthy or TSE-infected.

When the method is carried out in positional resolution, the sample (a thin slice of tissue applied to a microscopic slide) is placed in the beam path of an infrared microscope. The spectra can be taken by transmission or reflection in the infrared spectrometric arrangement. Infrared spectra are taken from various tissue areas. Positional resolution can be determined by the increment between measuring positions. It is very advantageous to use a computer-controlled X-Y stage that facilitates automated spectral measurements according to a freely selectable grid with defined increments. Such X-Y stages are standard accessories of state-of-the-art IR microscopes.

The result of a measurement in position resolution (mapping) is a series of infrared spectra wherein each spectrum represents a pixel on a fictitious grid of the thin slice of tissue. In this way, IR data is obtained that completely covers the selected area of the thin slice of tissue. The result of a measurement in position resolution (mapping) is a series of infrared spectra wherein each spectrum represents a pixel on a fictitious grid of the thin slice of tissue. In this way, IR data is obtained that completely covers the selected area of the thin slice of tissue. Position-specific information about the expansion of TSE in the tissue is maintained by comparing each mapping record with the reference database and thus classifying it as being healthy or infected. The following examples are to illustrate the way in which CNS samples obtained from scrapie-infected hamsters can be distinguished from samples obtained from healthy control animals based on the disease-specific modifications in their infrared spectra using the method according to the invention.

Example 1

Adult female Syrian hamsters *(Mesocricetus auratus)* were intracerebrally and intraperitoneally infected with the 263K scrapie strain (provided by Dr. Richard Kimberlin). In the terminal stage of the disease (70 to 120 days after infection), the brains of these animals (S) and of matching non-infected control animals were removed post mortem; corresponding pairs for comparison were of similar age. Small pieces (μg-scale amounts) of the natively resected medulla oblongata and Varolian pons were put into an FTIR cuvet equipped with CaF_2 windows and an optical path length of 8 μm (thickness of layer). The infrared spectra of these samples were measured in transmission/absorption using an FTIR spectrometer (spectral resolution: 4 cm^{-1}, apodization: Happ-Genzel, number of scans: 126, zero filling: 4). Two typical spectra of S- and N-tissue samples are shown in Fig. 2 in the spectral region from 1300 to 1000 cm^{-1} in which differences that can be observed are par-

ticularly prominent. The second derivations are represented for improved visualization of the bands, showing the band peaks as minima.

Example 2

In a variation of the embodiment described in Example 1, 10 S- and 10 N-samples obtained as in Example 1 were homogenized in H_20 (10 µl of H_20 per mg of tissue material). Aliquots of 35 µl of the suspensions were applied to a PC-controlled multiple sample holder made of ZnSe that is also suitable for measuring microbial samples (Helm et al. (1991) *J. Gen. Microbiol.* 137:69-79; Heim et al. (1991) *J. Microbiol. Meth.* 14:127-142; Neumann (1998) *Proc. SPIE* 3257:245-257), and dried as described in the literature. The infrared transmission spectra of the films thus obtained were taken and classified hierarchically based on second derivations of these spectra in the spectral region from 1100 to 1000 cm^{-1}. Fig. 3 shows the dendrogram of this spectrum classification as calculated using Ward's algorithm. The spectra of infected animals (S-3 through S-10) could be perfectly distinguished from those of the healthy animals (N-1 through N-10).

Example 3

In a variation of the embodiments described in Examples 1 and 2, cryostat sections were created of CNS samples obtained from N- and S-animals as described above, and measured and characterized using the generally known method of FTIR mapping (Diem et al. (1999) Appl. Spectroscopy 53: 148A-160A; Lasch & Neumann (1998) *Cell. Mol. Biol.* 44; 189-202; Choo et al. (1996) *Biophys. J.* 71:1672-1679) and infrared imaging (Lasch & Neumann (1998) *Cell. Mol. Biol.* 44:189-202; Lasch et al. (1998) *Proc. SPIE* 3257:187-198). Spectra were taken of 1.5 mm by 1.5 mm areas in increments of 50 µm through an aper-

ture of 60 µm. The spectra obtained from the S- and N-samples were first separately classified in hierarchies to differentiate the typical spectra for the various brain structures. Figure 4A shows a dendrogram that was calculated after data compression using principal component analysis based on the first three principal components between 1450 and 950 cm^{-1} (ca. 500 data points). The four main classes can be assigned to the four histologically defined cerebral structures: molecular layer, ganglion cell layer, granular cell layer, and white substance matter. In addition, nine spectral classes (numbered 1 to 9) were separated that correspond to specific structures within the cerebellum. Fig. 4A only shows each third spectrum of the mapping record that contains 930 spectra for better clarity. Subsequently, the spectra of corresponding spectral classes (e.g. class 2 of the molecular layer spectra - the gray substance of the cerebellum) of the N- and S-samples were compared. The upper part of Figure 4B (a) compares normed vector second derivations of sample spectra from scrapie-infected animals (dashed line) and from healthy animals (solid line). The lower part shows differential spectra of normed vector S- and N-spectra from a) for the respective tissue structures. All spectra used for this comparison are averages of spectra of a spectral class A, figure 4A). They are identified by the name of their cerebellar layer and the number of their spectral class. The characteristic spectral differences observed for each tissue class are suitable for reliably diagnosing the disease-associated pathogenetic process.

We claim:

1. A method for diagnosing TSE-induced pathologic changes in tissues, said changes being caused by scrapie, BSE or another disease of the TSE group of diseases, characterized in that

(a) infrared radiation is directed onto a tissue sample showing pathologic changes due to TSE, and that the spectral characteristics after interaction with the sample are recorded, and

(b) the infrared spectra thus obtained are compared and classified against a reference database that contains infrared spectra of TSE-infected and non-infected tissues.

2. The method according to claim 1, characterized in that said tissue sample was collected from the central nervous system, the peripheral nervous system, or from organs of the lymphatic system, the digestive systems, the endocrine system, the cardiovascular system, or the respiratory system.

3. The method according to at least one of claims 1 and 2, characterized in that said infrared spectrum of the tissue is measured either in one or several regions of the mid-infrared range from 500 to 4000 cm^{-1} or the near infrared range from 4000 to 10000 cm^{-1}, or in both regions.

4. The method according to at least one of claims 1 through 3, characterized in that said infrared spectrum of the tissue is measured in the spectral region from 1000 to 1300 cm^{-1} of the mid-infrared range.

5. The method according to at least one of claims 1 through 4, characterized in that said infrared radiation interacts with said sample, and the characteristically altered radiation is detected, in a transmission/absorption, attenuated total reflection, direct or diffuse reflection measuring setup, or by using IR waveguides.

6. The method according to at least one of claims 1 through 5, characterized in that said infrared spectrum of the sample to be examined is compared against the reference database using one or several methods of pattern recognition, preferably algorithms of multivariate statistics or artificial neuronal networks, and that the spectral regions said comparison is based on are determined using methods for extracting optimum spectral characteristics, such as genetic algorithms.

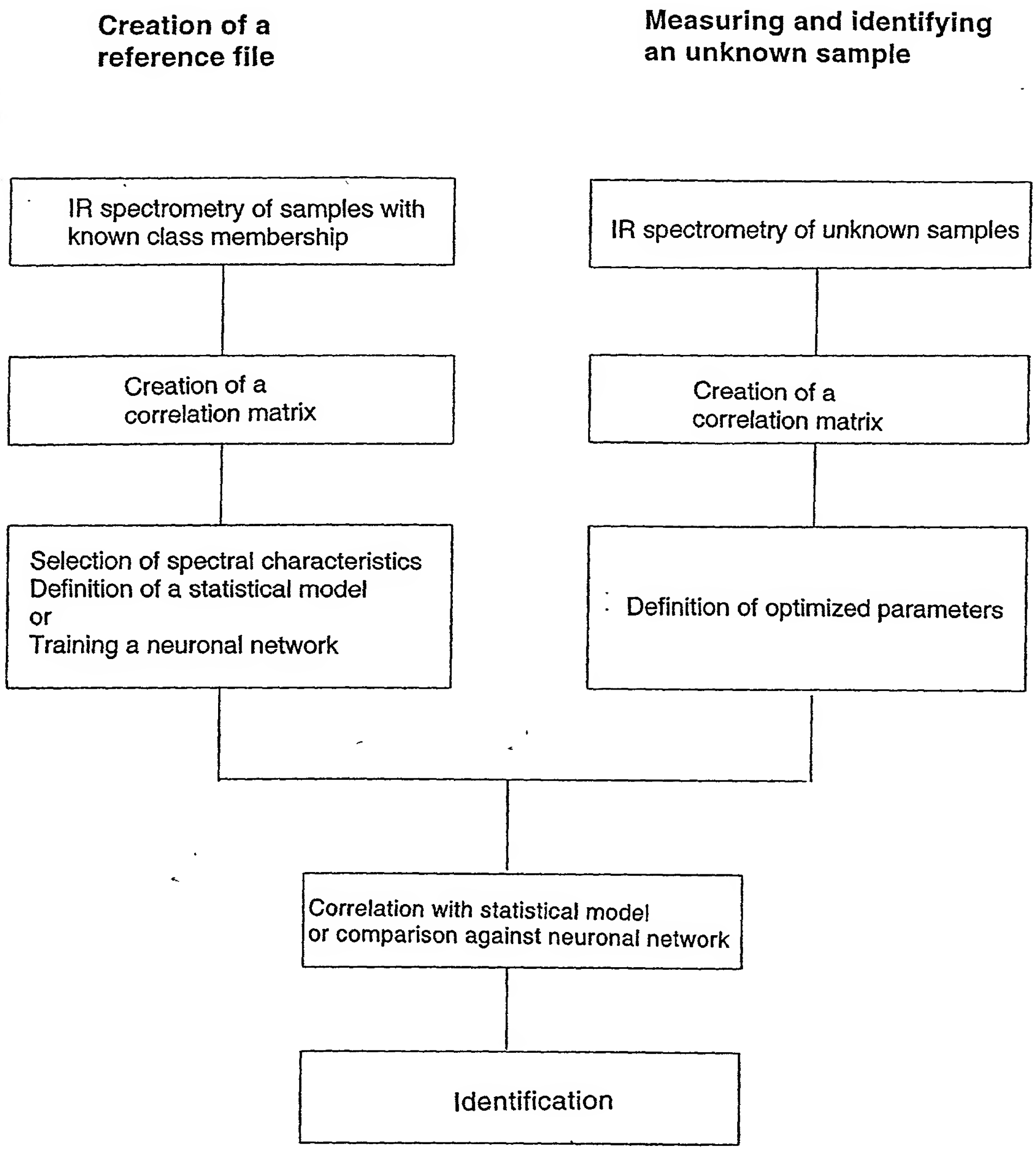
7. The method according to at least one of claims 1 through 6, characterized in that said infrared spectrum is measured on a thin slice of tissue using an IR microscope set up for transmission or direct reflection spectrometry.

8. The method according to claim 7, characterized in that said infrared spectra are measured in positional resolution, i.e. mapped to the tissue site where the infrared beam is transmitted through the sample.

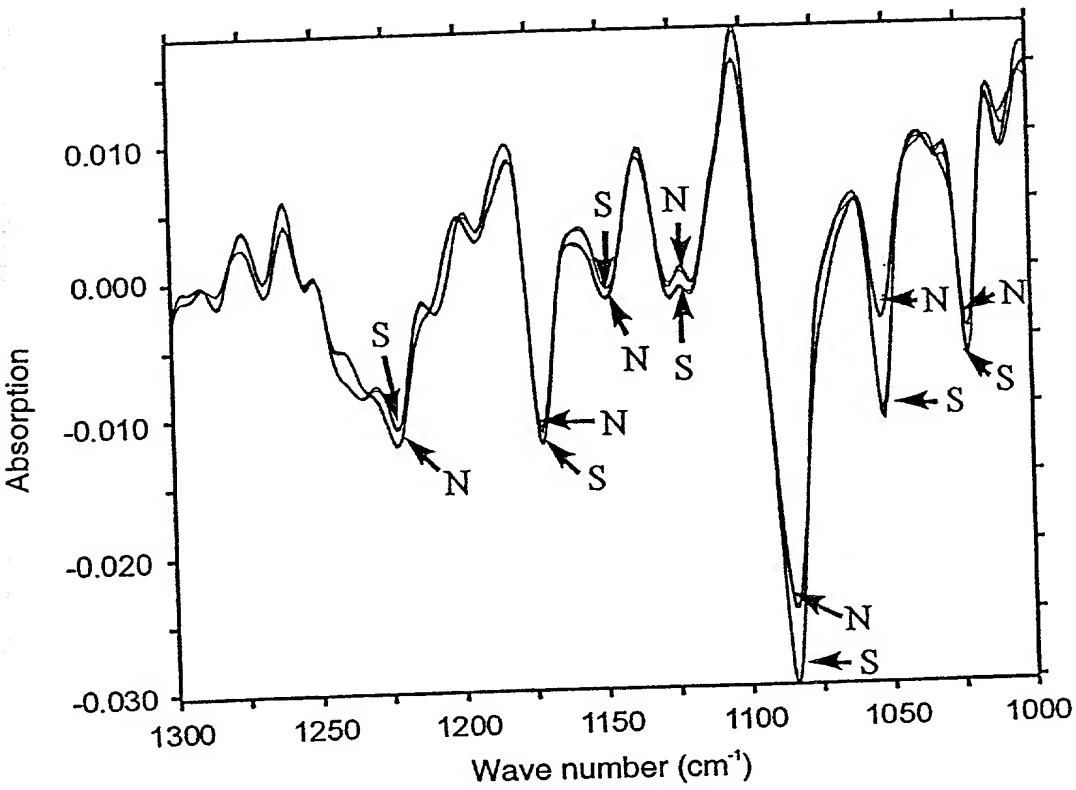
9. The method according to at least one of claims 7 and 8, characterized in that each mapped infrared spectrum is compared against the reference database, thereby providing localized information on the spread of the disease in the tissue.

10. The method according to at least one of claims 7 through 9, characterized in that said reference database contains reference spectra of TSE-infected tissues and non-infected tissues of all structures that can be distinguished in the tissue section using infrared spectroscopy.

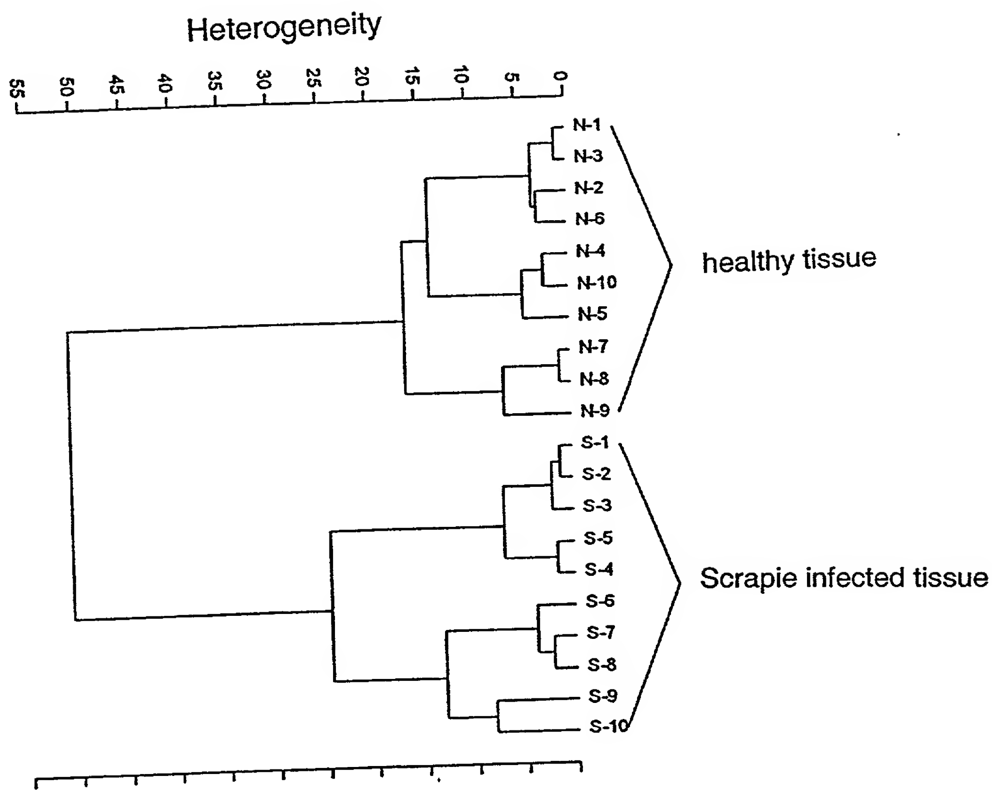
Figur 1



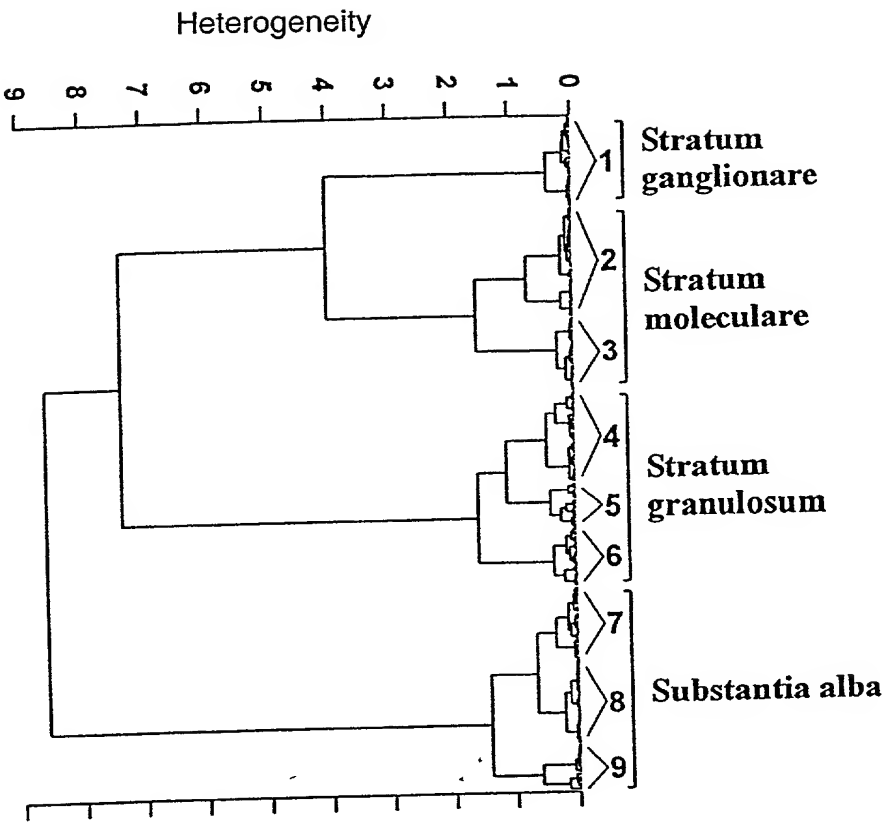
Figur 2



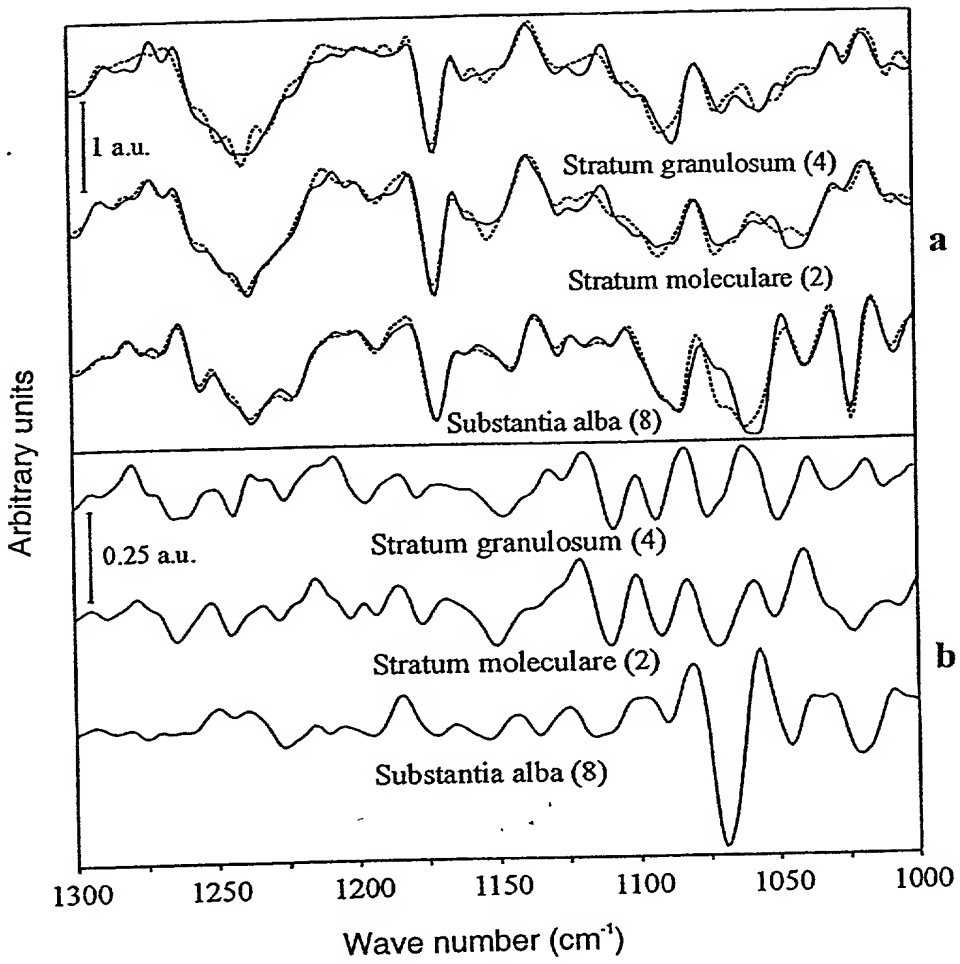
Figur 3



Figur 4A



Figur 4B


1 a.u.
Stratum granulosum (4)
Stratum moleculare (2)
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a
Arbitrary units
0.25 a.u.
Stratum granulosum (4)
Stratum moleculare (2)
Substantia alba (8)
b
1300
1250
1200
1150
1100
1050
1000
Wave number (cm-1)

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Method For Diagnosing TSE-Induced Changes In Tissues Using Infrared Spectroscopy,

the specification of which

(check one)

☐ is attached hereto.

☒ was received on November 8, 2001 as

☐ Application Serial No. 10/009,226

and was amended on November 8, 2001 (if applicable).

☒ was filed as PCT international application

No. PCT/DE00/01404 on 03 May 2000

and was amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
199 23 811.1 (Number)	Germany (Country)	20 May 1999 (Day/Month/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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